Tree Physiology 24, 729–736

Transgenic poplar characterized by ectopic expression of a pine cytosolic glutamine synthetase gene exhibits enhanced tolerance to water stress

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Received July 14, 2003; accepted November 15, 2003; published online May 3, 2004

Summary Physiological responses to water stress in hybrid poplar (INRA 7171-B4, *Populus tremula* L . × *P. alba* L.) lines transformed to overexpress a pine cytosolic glutamine synthetase (GS1) gene were compared with those of non-transgenic plants. Before, during and after a drought treatment, net photosynthetic rates (A_{net}) were higher in transgenic than in non-transgenic plants. Stomatal conductance (g_s) was higher in transgenic than in non-transgenic plants before, but not after exposure to drought. Before drought treatment, a sudden reduction in photosynthetic photon flux caused a greater burst of CO_2 efflux in transgenic than non-transgenic plants, indicating greater photorespiratory activity. Drought caused greater reductions in photochemical quenching, photosystem II (PSII) antennae transfer efficiency (F_v'/F_m') and light-adapted PSII yield (ϕ_{PSII}) in non-transgenic than in transgenic plants, especially at low irradiances. Antennae-based thermal dissipation was higher in transgenic plants than in non-transgenic plants both during the imposition of drought and 1 or 3 days after the relief of drought. Under severe water stress and subsequently, transgenic plants maintained a higher expression of glutamine synthetase, glutamate synthase and Rubisco and higher concentrations of chlorophyll and glycine than non-transgenic plants. These findings indicate that overexpression of pine cytosolic GS1 enhanced sustained photosynthetic electron transport capacity during severe stomatal limitation. The data also suggest that ectopic expression of cytosolic GS increases photorespiratory activity, and that this serves as a protective sink for electrons from photosynthetic reaction centers.

Keywords: drought, fluorescence, photorespiration, photosynthesis.

Introduction

Nitrogen (N) assimilation is central to the process of growth in woody plant species (Kozlowski and Pallardy 1997). In addition to determining the allocation dynamics of limiting nitrogen resources, components of the N-assimilatory pathway contribute to the ability of plants to tolerate environmental stress (Leustek and Kirby 1990). Plant nitrogen status has been linked to photosynthetic capacity and plant water use (Kruger and Reich 1997, Davis et al. 1999). In general, tissue N content shows a strong linear relationship with photosynthetic capacity, both within and between species (Reich et al. 1998), and plants with a high photosynthetic capacity are usually able to deploy rapidly reversible photoprotective mechanisms (i.e., dynamic photoinhibition) (Demmig-Adams and Adams 1992, Osmond 1994).

The key N-assimilation enzyme, glutamine synthetase (GS) regulates the pool size of organic molecules linking the carbon and nitrogen assimilation pathways that are critical components of photorespiration (Leegood et al. 1995). Photorespiration may provide an alternative sink for electrons from photosynthetic reaction centers under CO_2-limiting conditions, such as under water stress as a result of stomatal closure (Osmond and Grace 1995). Consistent with a role of GS in linking plant nitrogen status with the capacity for dynamic photoinhibition, it has been demonstrated that tobacco transgenically altered to overexpress chloroplastic GS showed less irreversible photoprotective down-regulation of photosynthesis than non-transgenic plants (Kozaki and Takeba 1996).

Genetic transformation may provide a means to increase the intrinsic efficiency of economically important plants to acquire and assimilate limiting nutrients on nutrient-poor sites or to utilize mineral resources more efficiently under favorable conditions. Havaux et al. (1988) found that hybrid poplar lines varied in tolerance to water stress, and suggested that selection of hybrid lines for this or other specific ecophysiological traits could increase production. Genetic transformation provides a rapid means of introducing such characteristics.

Transformation of hybrid poplar to increase glutamine production by overexpression of the pine cytosolic glutamine synthetase (GS1) gene resulted in a 75% increase in early vegetative growth and was accompanied by an increase in chloro-

phyll content and total soluble protein (Gallardo et al. 1999, Fu et al. 2003). However, the reasons for enhanced growth of transgenic lines remain unclear. An increase in available glutamine has been associated with increased resistance to water stress (Leustek and Kirby 1990). Hoshida et al. (2000) demonstrated that overexpression of chloroplastic GS in transgenic rice increased photorespiratory capacity and enhanced tolerance to salt stress. These findings suggest a role of photorespiration in protection against photoinhibition under stressful conditions. Moreover, GS controls the rate-limiting step in photorespiration: the reassimilation of ammonium generated by photorespiration (Kozaki and Takeba 1996). Furthermore, GS has a significant effect on the biosynthesis of nitrogen-containing compounds, including amino acids and chlorophyll (Gallardo et al. 1999, Fu et al. 2003).

In this study, we compared the ecophysiological performance of transgenic poplars overexpressing the pine GS1 gene with non-transgenic plants before, during and after the transient imposition of water stress. Specifically, we hypothesized that, compared with non-transgenic plants, transgenic lines will: (1) have higher photosynthetic capacities and stomatal conductances; (2) show greater photorespiratory capacities; and (3) display less photoinhibitiory down-regulation of PSII function. We also hypothesized that, compared with non-transgenic plants, transgenic lines will maintain greater expression of GS, glutamate synthase (GOGAT) and Rubisco, and higher concentrations of chlorophyll and glycine during and after water stress.

Materials and methods

Plant material

We studied a hybrid poplar clone (INRA 7171-B4, *Populus tremula* L. × *P. alba* L.) and fast and medium growth transgenic lines derived from this clone that overexpress the pine cytosolic glutamine synthetase (GS1) gene (Gallardo et al. 1999). Rooted cuttings (9–12 months old) were planted in 6-inch pots containing a peat-based commercial growth medium (Metro-Mix 200, Scotts, Marysville, OH) without supplementary nutrients and raised in a growth chamber supplying a 16-h photoperiod (24–26 °C). Photosynthetic photon flux (PPF) was measured at the leaf-level with a Li-Cor 190 quantum sensor (Li-Cor, Lincoln, NE) every 15 min throughout the 16-h photoperiod, and ranged from 295 to 330 μmol m^{-2} s^{-1}. Three replicate samples of each plant type were sampled for photosynthetic gas exchange and chlorophyll fluorescence measurements during a drought and post-drought treatment period.

Water status

Soil water potential was measured with a WP4 dewpoint potential meter (Decagon Devices, Pullman, WA). Soil water potential and soil wet mass (four samples) were recorded every hour for 7 h. Soil samples were weighed after drying overnight at 60 °C and volumetric soil water (θ) calculated. Nonlinear regression (SigmaPlot v4.01, SPSS, Chicago, IL) was used to relate θ to soil water potential (ψ_{soil}): $\psi_{soil} = 0.9031 + 1.305\ln(\theta - 0.1081)$ ($R^2 = 0.97$; $P < 0.0001$). This allowed conversion of θ, estimated with a time-domaine-reflectrometry (TDR) soil moisture meter (Theta Meter, Delta-T Devices, Cambridge, U.K.), to track changes in soil water throughout the experiment. We used soil water potential as a proxy measure of plant water status, because the study plants were small and continued harvesting of leaves would likely have significantly affected plant source–sink relationships and, hence, photosynthetic activity (Thomas and Strain 1991).

Stress and recovery treatment

Water stress was applied to plants by withholding irrigation for 8 days, by which time θ was between 15 and 20%, equivalent to a soil water potential of between –2 and –3 MPa. After the drought treatment, plants were watered every day for 5 days, until θ was between 50 and 55%, equivalent to a soil water potential of between –1 and 0 MPa.

Gas exchange measurements

Photosynthetic gas exchange measurements were made with an open flow LI-6400 portable photosynthetic system (Li-Cor, Lincoln, NE). Leaf tissue (6 cm^2) was enclosed in the cuvette and exposed to 2000 μmol m^{-2} s^{-1} PPF from a red/blue LED light source attached to the cuvette. Leaf temperature, cuvette CO_2 concentration, and leaf-to-atmosphere vapor pressure were maintained at 25 °C, 400 ppm, and 0.1 to 0.22 kPa, respectively. Leaves were allowed to equilibrate to cuvette conditions for a minimum of 3 min before four measurements of net assimilation (A_{net}) and stomatal conductance to water vapor (g_s) were made. Following these measurements, PPF was reduced to 200 μmol m^{-2} s^{-1}, and A_{net} and g_s were recorded every minute for 6 min. This protocol was followed to assess any post-lower illumination burst (PLIB) of CO_2, an indicator of photorespiratory capacity (Vines et al. 1983).

Fluorescence measurements

Individual leaves were dark-adapted for a minimum of 45 min, after which chlorophyll fluorescence parameters were estimated with a pulse-amplitude-modulated (PAM) fluorimeter (Hansatech FMS 2, Hansatech Instruments, Kings Lynn, U.K.). Baseline fluorescence (F_0) was established by averaging fluorescence yield over 2 s after a 30-s exposure to sub-saturating light at a modulation setting of 2 and a relative gain of 50. The leaf was then exposed to a saturating PPF (18,000 μmol m^{-2} s^{-1}) for 0.7 s to establish maximum fluorescence yield (F_m). Optimal photochemical yield of PSII (F_v/F_m) was calculated as $F_v/F_m = (F_m - F_0)/F_m$. Following F_v/F_m estimation, actinic light was increased from a PPF of 4.5 μmol m^{-2} s^{-1} to 20, 85, 350, 900 and 2000 μmol m^{-2} s^{-1}. After a 40-s exposure to each irradiance, light-adapted steady-state fluorescence yield (F_s) was averaged over 2 s, followed by exposure to saturating light for 0.7 s to establish F_m'. The sample was then shaded for 5 s with a far-red light source to determine F_0', which was used for correct determination of photochemical quenching ($qP = (F_m' - F_s)/(F_m' - F_0')$), thermal dissipation

(NPQ = $(F_m - F_m')/F_m'$), antennae transfer efficiency of photosystem II (PSII) ($F_v'/F_m' = (F_m' - F_0')/F_m'$), and light-adapted quantum yield of PSII ($\phi_{PSII} = (F_m' - F_s)/F_m'$).

Protein extraction

One gram of leaves (fourth from the apex) was ground with sand (0.5 g) and 4 ml of extraction buffer (50 mM Tris-HCl, 2 mM EDTA, 10 mM 2-mercaptoethanol, 10% (v/v) glycerol, pH 8.0) with a mortar and pestle. Extracts were centrifuged at 10,000 *g* for 15 min at 4 °C and the supernatant used for protein determination, electrophoresis, and immunoblot detection. Protein content was estimated by the Bradford method (Bradford 1976) with a bovine serum albumin standard.

Electrophoresis and immunoblot detection of GS, GOGAT and large subunit of Rubisco polypeptides

Total protein was prepared from leaves, excluding midribs, as described previously (Gallardo et al. 1999) and separated by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) (10% (w/v) acrylamide) in the discontinuous buffer system of Laemmli (1970) at 25 mA for 1.5 h. For comparison, total proteins from pine cotyledons were also studied. Equal amounts of protein (20 µg) were loaded in each lane and the gels were stained with Coomassie blue. Proteins were electro-transferred to nitrocellulose filters, and specific polypeptides, including GS, Fd-GOGAT and the large subunit of Rubisco, were detected with polyclonal antibodies as described previously (García-Gutiérrez et al. 1993, 1995, Cantón et al. 1996, Gallardo et al. 1999). Immuno complexes were detected with a solution of 16.9% (v/v) 4-chloro-1-naphthol in ethanol, 83% (v/v) TBS (20 mM Tris-HCl and 150 mM NaCl at pH 7.5) and 0.08% (v/v) H_2O_2.

Determination of free glycine

Four-mm diameter discs were cut from three to four fresh leaves (between the third and sixth from the apex). Two hundred milligrams of discs were put in a 2-ml Eppendorf tube containing 0.8 ml of 5% (v/v) perchloric acid. Samples were frozen and thawed three times, centrifuged and the supernatants analyzed for glycine (Minocha et al. 1994). Data from transgenic lines and replicates of each line were pooled to minimize variation.

Chlorophyll determination

Chlorophyll was extracted from leaves (fourth from the apex) by grinding in 80% (v/v) acetone with a pestle and mortar. Chlorophyll was determined spectrophotometrically (Graan and Ort 1984).

Statistical analysis

A repeated measures split-plot analysis of variance (ANOVA, Statistic v. 4.0, Analytical Software, St. Paul, MN) was undertaken to assess the effects of genetic transformation, during exposure to drought and during subsequent recovery. The whole-plot factor was tree type (transgenic versus control). The sub-plot factors were the four sampling periods (pre-, full stress, 24-h and 3-day recovery), sampling period (in the case of gas exchange) and irradiance (in the case of fluorescence measurements). Pairwise means comparisons were made by the least significant difference method (LSD), and α-adjusted general linear contrasts (*t*-test) were used to test specific post-hoc comparisons contributing to any higher-order interactions. All photochemical quenching (qP), F_v'/F_m' and light-adapted photosystem II yield (ϕ_{PSII}) data were arcsine-transformed to meet ANOVA data distribution assumptions (Zar 1974). Chlorophyll and glycine data were analyzed by a two-way repeated-measures ANOVA. Pairwise comparisons of main effects (plant type or time) were made with α-adjusted LSD ($P < 0.05$).

Results

Gas exchange

Photosynthetic gas exchange responses showed a significant type-by-time interaction in net assimilation ($F = 6.33$; $P < 0.05$) and stomatal conductance ($F = 14.16$; $P < 0.05$), suggesting that drought responses of transgenically altered poplar lines differed from those of control plants (Figure 1). Before stress, A_{net} was 20% higher (6.35 ± 0.282 µmol m^{-2} s^{-1} (± SE)) in the high-performance transgenically altered line than in the medium-performance transgenic and control lines (5.00 ± 0.385 and 4.73 ± 0.39 µmol m^{-2} s^{-1}, respectively) ($t = -6.00$; $P < 0.0001$; Figure 1). Exposure to lower PPF (200 µmol m^{-2} s^{-1}) caused a PLIB of CO_2 (Vines et al. 1983) in both transgenic plants and controls. On recovery from PLIB, A_{net} in transgenic plants recovered to rates above those of controls (Figure 1). After about 8 days without water (θ = 15%, θ_{soil} = −3 MPa), and 1 and 3 days after rewatering (θ = 50%, θ_{soil} = −1.0 MPa), there were no detectable differences in soil water potentials or volumetric water contents between transgenic and control plants. Water stress sharply reduced A_{net}, which remained low after 1 and 3 days of recovery (Figure 1). Despite similar soil water availabilities, A_{net} in the high- and medium-performance transgenic lines was nearly 65% higher across all irradiances (2.63 ± 0.138 and 2.57 ± 0.109 µmol m^{-2} s^{-1} in high- and medium-performance transgenic lines, respectively) than in control plants (0.98 ± 0.194 µmol m^{-2} s^{-1}) across this time period ($t = 8.57$; $P < 0.0001$) (Figure 1). Before the imposition of stress, g_s was higher in the high-performance transgenic line (0.284 ± 0.282 mol m^{-2} s^{-1} SE) than in the medium-performance and control lines (0.143 ± 0.012 and 0.123 ± 0.011 mol m^{-2} s^{-1}, respectively, $t = -6.00$; $P < 0.0001$) (Figure 1). During water stress and pooled across 1 and 3 days of recovery, g_s tended to be lower in controls than in transgenic plants (Figure 1) ($t = -1.75$; $P = 0.0812$).

Chlorophyll fluorescence

Optimal PSII yield (F_v/F_m) for both transgenic lines exceeded those in non-transgenic plants. Pooled across the experiment, F_v/F_m for control poplar was about 17% lower (0.48 ± 0.08 SE) than that for medium- and high-performance trans-

genic lines, which had similar values (mean of 0.65 ± 0.03 for both lines). However, the difference between transgenic and non-transgenic plants was not statistically significant. All lines showed a significant 31% irreversible decline in F_v/F_m following drought and during recovery ($F = 35.43$; $P < 0.05$), with no type-by-time interaction effect.

All chlorophyll fluorescence parameters responded to changes in irradiance and drought, depending on plant type. Plant type-specific light responses were apparent in qP ($F_{12,161} = 4.29$; $P < 0.05$), antennae-based thermal dissipation (NPQ) ($F = 1.77$; $P < 0.1$), F_v'/F_m' ($F = 4.81$; $P < 0.05$) and ϕ_{PSII} ($F = 6.14$; $P < 0.05$). These responses were associated with distinct differences between transgenic and non-transgenic plants at low and high irradiances. In low light, light saturation kinetics of transgenic plants exceeded values for non-transgenic plants for qP (Figure 2), F_v'/F_m' (Figure 3) and ϕ_{PSII} (Figure 4) ($t = -6.22$, -8.04 and -8.79, $P < 0.05$ for qP, F_v'/F_m' and ϕ_{PSII}, respectively). However, NPQ did not differ between plant types in low light (Figure 5). In saturating light, qP was significantly lower in transgenics than non-transgenic plants ($t = 2.68$; $P = 0.008$; Figure 2). In contrast, values of NPQ and F_v'/F_m' of transgenic lines exceeded those of non-transgenic plants (Figures 5 and 4, respectively); $t = -5.55$, -1.98; $P < 0.05$, respectively), whereas ϕ_{PSII} values were indistinguishable between plant types at saturating irradiances (Figure 4).

Plant-type specific responses to drought were apparent in the energy-dependent parameters NPQ ($F_{6,161} = 1.84$; $P < 0.1$) and F_v'/F_m' ($F_{6,161} = 6.25$; $P < 0.05$). Before water stress, NPQ and F_v'/F_m' did not differ between plant types (Figures 5 and 3, respectively). At peak stress and 24 h after recovery, NPQ was significantly greater in the transgenic lines than in non-transgenic plants ($t = -3.41$; $P = 0.0008$), as was the case after 3 days of recovery ($t = -3.5$; $P = 0.0006$) (Figure 5). In addition, NPQ showed a general increase after 3 days of recovery compared with values at maximum water stress and 1 day after the resumption of irrigation ($t = -5.38$; $P < 0.0001$) (Figure 5). Similar dynamics occurred in F_v'/F_m', with higher F_v'/F_m' in transgenic lines compared with controls at maximum stress and after 1 day of recovery ($t = -5.77$; $P < 0.0001$), and after 3 days of recovery ($t = 4.98$; $P < 0.0001$), but with no overall increase after 3 days of recovery (Figure 3).

Enzyme expression

Relative expression of GS, and for comparison, of glutamate synthase (GOGAT) and the large subunit of Rubisco (LSU), in transgenic lines and controls during the stress experiment, were determined by immunoblotting. Immunoblots showed that, before water stress, expression of GS1 and GS2 in mesophyll was low in control plants compared with transgenic plants (Figure 6). During water stress and recovery, the expression of GS1 and GS2 in control plants was significantly reduced. Before water stress, both the chloroplastic GS2 and the pine cytosolic GS1 (transgene) polypeptides were present and markedly higher in the transgenic lines than in control plants (Figure 6). During water stress, the expression of both GS2 and GS1 decreased, but the expression of both was much higher than in the controls. After 5 days of recovery from water stress, there was an increase in the expression of both GS1 and GS2 in transgenic lines, but expression did not reach pre-drought values. In the controls, GOGAT expression decreased with the onset of water stress and reached a minimum during the 5-day recovery period. Transgenic lines maintained expression of GOGAT during water stress and recovery. A significant decrease in the expression of LSU was observed in non-transgenic controls during and after water stress compared with pre-stress values, whereas transgenic lines maintained expression of LSU during water stress and recovery.

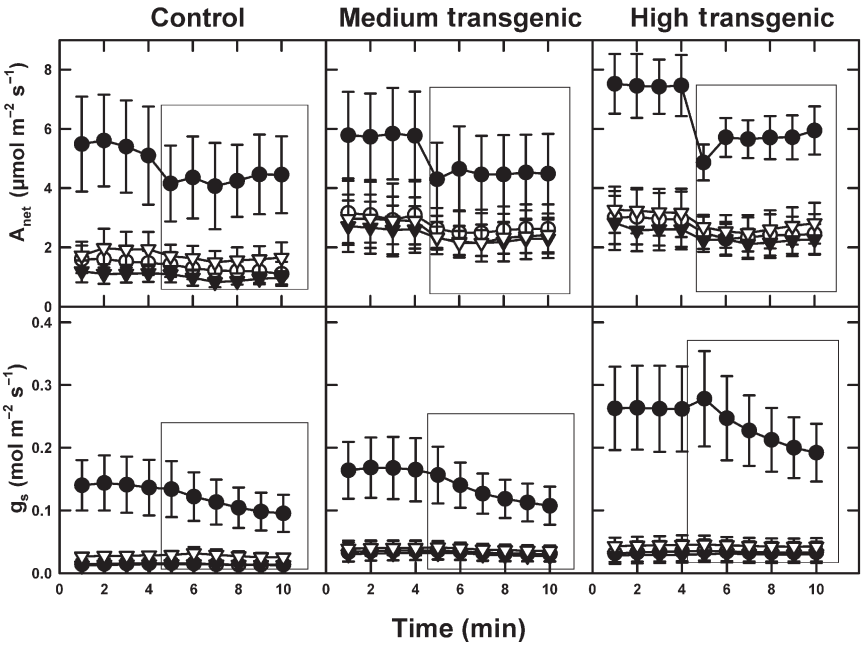

Figure 1. Net photosynthesis (A_{net}) and stomatal conductances (g_s) of non-transgenic and medium- and high-performance transgenic poplar lines at irradiances of 2000 (outer box) and 200 (inner box) μmol m^{-2} s^{-1} before the imposition of drought (●), at maximum water stress (○), and 1 (▼) and 3 days (▽) after the termination of drought. Each value is the mean of three independent measurements. Bars indicate ± 1 SE.

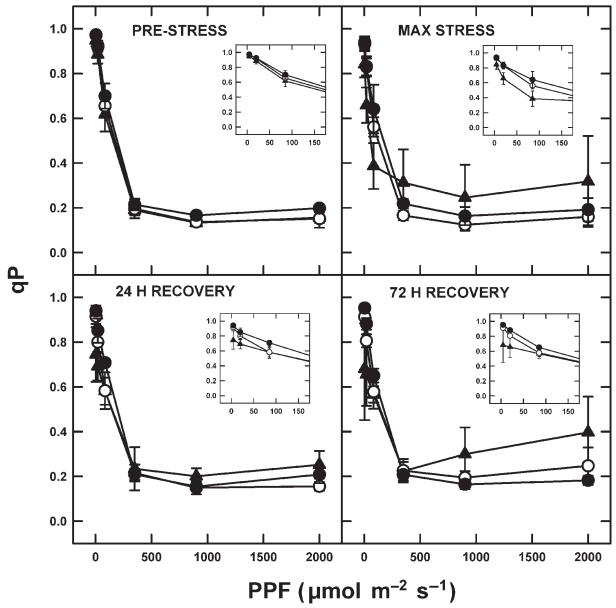

Figure 2. Light response curves of photochemical quenching (qP) of non-transgenic (▲) and medium-performance (○) and high-performance (●) transgenic poplar lines before drought exposure, at maximum water stress, and 1 and 3 days after the termination of drought. Inset graphs show low photosynthetic photon flux (PPF) responses (0–175 μmol m^{-2} s^{-1}). Each value is the mean of three independent measurements. Bars indicate ± 1 SE.

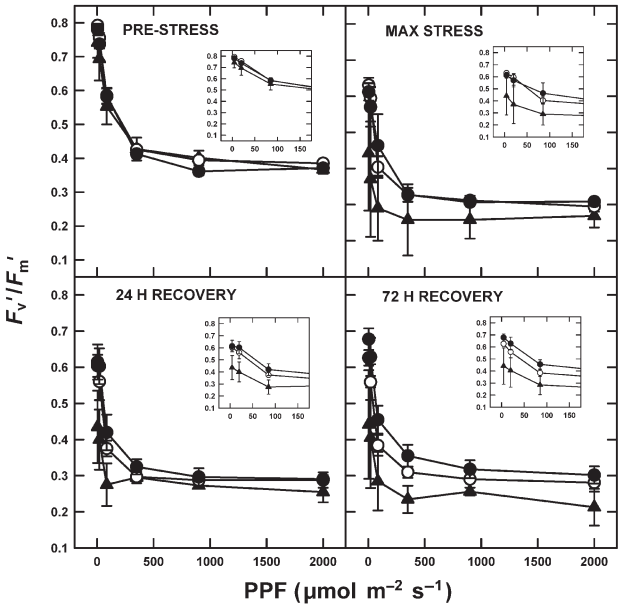

Figure 3. Light response curves of antennae efficiency of photosystem II (F_v'/F_m') of control (▲) and medium-performance (○) and high-performance (●) transgenic poplar lines before the imposition of drought, at maximum water stress, and 1 and 3 days after the termination of drought. Inset graphs show low photosynthetic photon flux (PPF) responses (0–175 μmol m^{-2} s^{-1}). Each value is the mean of three independent measurements. Bars indicate ± 1 SE.

Free glycine and chlorophyll concentrations

During water stress, transgenics had 38% higher glycine concentrations (65.06 ± 14.17 nmol g_{DM}^{-1}) than control plants (47.08 ± 16.52 nmol g_{DM}^{-1}). After 24 h of recovery, glycine concentrations were 62% higher in transgenic poplars (31.97 ± 5.53 nmol g_{DM}^{-1}) than in non-transgenic plants (19.78 ± 3.19 nmol g_{DM}^{-1}), but the differences between lines were not statistically significant. After 3 days of recovery, glycine in non-transgenic plants fell to low concentrations, whereas detectable concentrations of glycine (30 nmol g_{DM}^{-1}) were maintained in transgenic lines (Figure 7).

Transgenic poplars had significantly higher chlorophyll concentrations compared with non-transgenic plants throughout the experiment ($F = 16.53$; $P < 0.05$) with no type-by-time interaction (Figure 7). Chlorophyll concentrations were nearly 50% higher in two transgenic lines (5.36 ± 0.13 and 6.65 ± 0.61 mg g_{DM}^{-1}) than in non-transgenic plants (4.01 ± 0.23 mg g_{DM}^{-1}) before the imposition of water stress (Figure 7). Although water stress sharply reduced chlorophyll concentrations in both transgenic and non-transgenic lines, the transgenic lines (3.67 ± 0.10 and 3.98 ± 0.33 mg g_{DM}^{-1}) maintained 59% higher chlorophyll concentrations than control plants (2.41 ± 0.45 mg g_{DM}^{-1}). Both transgenic and non-transgenic plants increased their chlorophyll concentration during recovery from drought compared with concentrations during maximum stress. After 24 h of recovery, chlorophyll concentrations in both transgenic lines (4.45 ± 0.23 and 4.16 ± 0.72 mg g_{DM}^{-1}) were about 62% higher than in control poplars (2.65 ± 0.50 mg g_{DM}^{-1}). In addition, after 3 days of recovery, transgenic lines had 42% higher chlorophyll concentrations (4.36 ± 0.22 and 4.40 ± 0.49 mg g_{DM}^{-1}) than non-transgenic plants (3.08 ± 0.16 mg g_{DM}^{-1}). Neither transgenic nor non-transgenic poplar lines achieved their pre-drought chlorophyll concentrations during the post-drought period (Figure 7).

Discussion

We compared the ecophysiological performance of transgenic poplars overexpressing the pine GS1 gene with untransformed lines before, during, and after recovery from transient water stress. Net photosynthetic rate was higher in transgenic poplars throughout the experiment. There was evidence of higher photorespiratory activity in transgenic lines, as indicated by proportionally greater post-lower illumination burst after exposure to saturating PPF (Figure 1). Despite some variability in the data, stomatal conductances were also higher in transgenics than in controls before water stress, but declined to similar values during stress and recovery (Figure 1).

Fluorescence data showed that energy-dependent quenching of light energy in the antennae is a significant component of the stress response in these plants, especially in transgenic plants. This component appears to be most significant during stress recovery (Figures 2–5). The NPQ, a measure of thermal-dissipative photoprotection and short-term adjustment to irradiance, is highly dynamic and tightly coupled to xanthophyll cycle kinetics (Osmond 1994, Anderson et al. 1997).

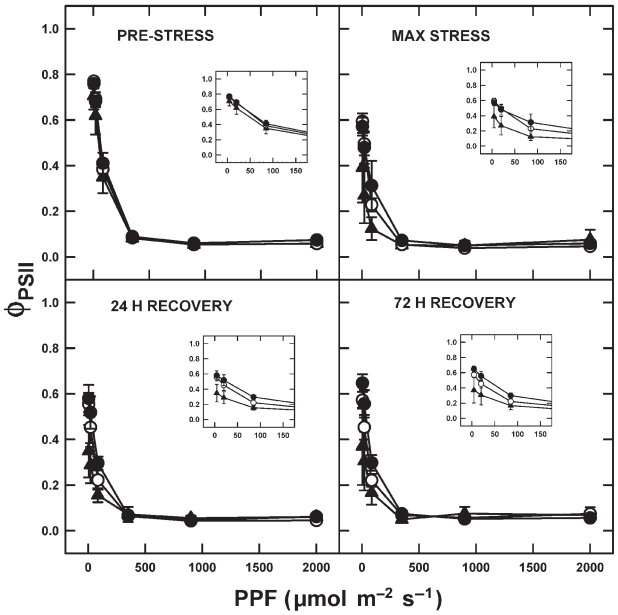

Figure 4. Light response curves of photosystem II quantum yield (ϕ_{PSII}) of non-transgenic (▲) and medium-performance (○) and high-performance (●) transgenic poplar lines before drought exposure, at maximum water stress, and after 1 and 3 days after the termination of drought. Inset graphs show low photosynthetic photon flux (PPF) responses (0–175 µmol m^{-2} s^{-1}). Each value is the mean of three independent measurements. Bars ± 1 SE.

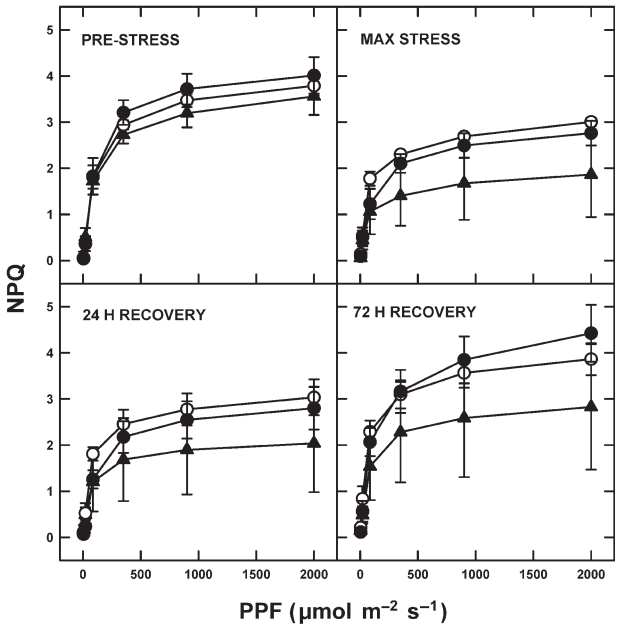

Figure 5. Light response curves of non-photochemical thermal dissipation (NPQ) of non-transgenic (▲) and medium-performance (○) and high-performance (●) transgenic poplar lines before the imposition of drought, at maximum water stress, and 1 and 3 days after the termination of drought. Each value is the mean of three independent measurements. Bars indicate ± 1 SE.

Both PSII antennae transfer efficiency (F_v'/F_m'), which is also an energy-dependent parameter (Baker 1994, Baker and Adams 1997), and light-adapted PSII yield (ϕ_{PSII}) remained higher in transgenic plants compared with controls throughout the recovery period. The F_v'/F_m' ratio is an index of the intrinsic efficiency of PSII under ambient light conditions, whereas ϕ_{PSII} directly reflects PSII efficiency (Baker and Adams 1997). Therefore, the varying responses of NPQ and F_v'/F_m' may reflect differences in fine- and course-scale adjustments in antennae-level thermal dissipation during drought recovery, resulting in PSII yields being higher in transgenic lines than in controls (Figure 5). Differences in qP between transgenic lines and controls likely affected ϕ_{PSII}, which is strongly determined by qP (Hymus et al. 1999). Higher PSII yield suggests greater potential for photochemical processes contributing to electron transport. Thus, our evidence suggests that overexpression of cytosolic GS in transgenic poplars results in better engagement of antennae-level, energy-dependent mechanisms to protect the reaction center (Osmond 1994).

Water stress also affected the expression of enzymes involved in ammonium assimilation and photosynthesis. Expression of GS1, GS2, Fd-GOGAT and the large subunit of Rubisco were diminished during imposition of water stress in both transgenic and control plants. However, control plants

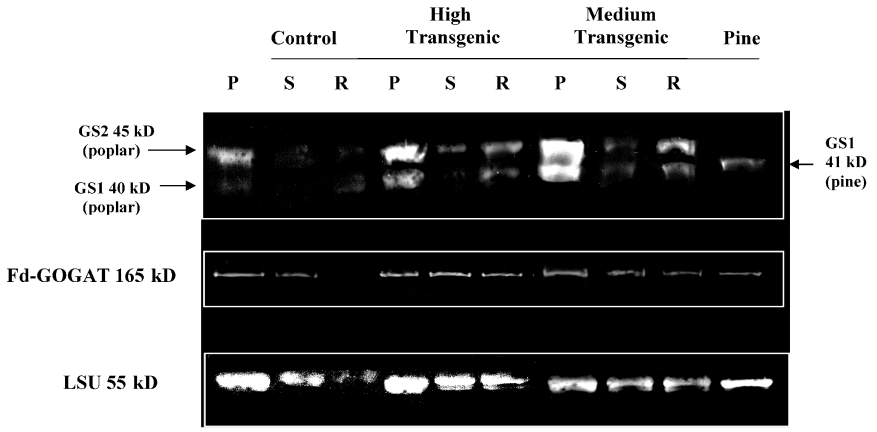

Figure 6. Western blot analysis of expression of glutamine synthetase (GS), glutamate synthase (Fd-GOGAT), and the large subunit of Rubisco (LSU) in leaves of non-transgenic and high- and medium-performance transgenic lines of poplar, before the imposition of drought (P), at maximum stress (S), and 5 days after the termination of drought (R).

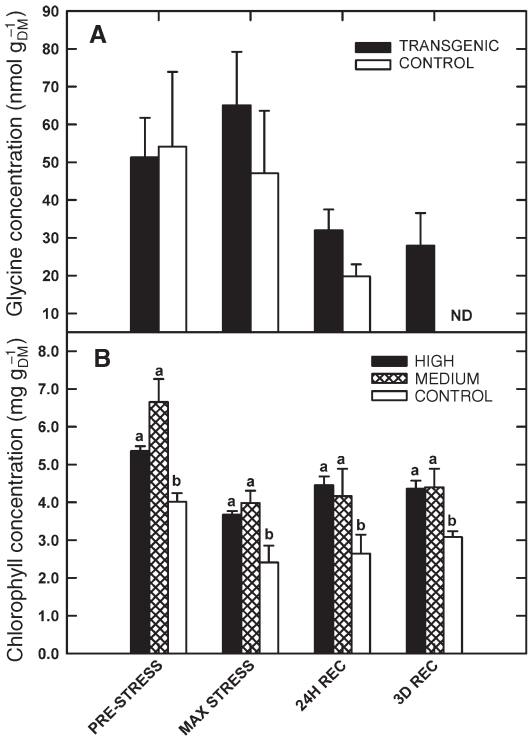

Figure 7. (A) Free glycine concentration in leaves of non-transgenic poplar and the mean free glycine concentration of multiple transgenic poplar lines. (B) chlorophyll content of non-transgenic and transgenic poplar lines before the imposition of drought, at maximum water stress, and 1 and 3 days after the termination of drought. Each value represents the mean of 4–5 independent measurements for glycine and three measurements for chlorophyll. Bars indicate ± 1 SE; letters differ significantly at $P < 0.05$ within a sampling period. Abbreviation: ND = not determined.

showed greater decreases in expression of these enzymes during and after water stress compared with transgenic lines, which maintained higher levels of enzyme expression throughout these periods (Figures 6). These data indicate that expression of fundamental leaf enzymes is higher in transgenic poplars overexpressing cytosolic GS than in untransformed controls, even under severe stress conditions.

The photoprotective role of photorespiration is controversial. Kozaki and Takeba (1996) showed that transgenic tobacco with twice the normal expression of chloroplastic GS displayed improved photorespiration capacity and increased tolerance to high light. These workers concluded that photorespiration protects against damage in high light. Hoshida et al. (2000) showed that transgenic rice overexpressing the chloroplastic GS displayed enhanced photorespiration capacity. Our fluorescence quenching data for transgenic poplars suggest that enhanced photorespiration could protect against over-reduction of the electron transport system (ET) during recovery from water stress. Additionally, Hoshida et al. (2000) concluded that the over-reduction of photosynthetic ET chains, which occurred under salt stress and during stomatal closure, could limit the CO_2 supply and activate photorespiration. In plants overexpressing GS, ammonium released during photorespiration could then be efficiently re-assimilated by the enhanced GS activity. Increased photorespiration produces phosphoglycerate and CO_2. These products can enter the Calvin cycle, consume NADPH and ATP, and result in a decrease in over-reduction in the ET chain. Moreover, higher photosynthetic rates can result in an increase in both dynamic and chronic photoinhibition (Osmond and Grace 1995). Finding an alternative for the diffusion of excess energy is a necessity, and a possible outlet could be photorespiration. Streb et al. (1998) proposed that photorespiration provides a strong electron sink and an important means for photoprotection in high light.

Although we did not measure photorespiration directly, we observed that overexpression of pine cytosolic GS in poplar was correlated with a greater post-lower illumination burst of photorespiratory CO_2 (Vines et al. 1983). This co-occurred with a greater capacity to protect against photooxidation (Figure 5). It is likely that photorespiration provides protection against photooxidation in transgenic poplar by providing an alternative pathway for light-induced electron transport, resulting in a higher quantum yield of PSII in transgenic than non-transgenic plants (Hoshida et al. 2000). Greater photorespiratory capacity in transgenic lines could also be important because a continued flow of electrons into photorespiration could insure that the trans-thylakoid pH gradient is maintained, thereby providing energy for greater antennae-level protection (Figures 3–5). This may also account for the higher A_{net} observed in transgenic plants after recovery from water stress, even under extreme stomatal limitation (Figure 1), and is consistent with higher F_v'/F_m' and ϕ_{PSII}, which may indicate greater ET capacity in transgenic plants. During recovery from water stress, when stomatal limitations are high (Figure 1), photorespiration may function as an energy sink in transgenic plants to sustain positive A_{net} (Figure 1).

Transgenic poplar lines maintained higher chlorophyll concentrations compared with the controls (Figure 7). These data support our assertions that GS transgenics display considerable capacity for energy-dependent quenching at the antenna level, especially after drought recovery (Figures 3 and 5). Furthermore, this dissipative capacity may help sustain higher realized PSII efficiency (Figure 5), and possibly higher electron transport capacity (Osmond and Grace 1995). Higher concentrations of free glycine in transgenic lines than in controls during and after water stress (Figure 7) provide further evidence for higher photorespiratory capacity in GS transgenics (Oliveira et al. 2002). This is in agreement with our gas exchange data (Figure 1). However, variation in glycine concentrations suggests that the impact of GS overexpression on photorespiratory activity is inconsistent. Given that photorespiration requires considerable coordination among multiple organelles and biochemical pathways (Osmond and Grace 1995, Oliveira et al. 2002), this variation is not unexpected. Rather, our results demonstrate that, during onset of severe drought stress and recovery from stress, GS overexpression results in a greater capacity to protect the critical initial steps of effective photosynthetic light harvesting. This strongly suggests that increased resource acquisition capacity in GS1 transgenic poplars al-

lows greater resource allocation to photoprotective mechanisms, thereby enhancing vegetative growth (Fu et al. 2003).

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